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Identification of Quantitative Trait Loci for Plant Height, Crown Diameter, and Plant Biomass in a Pseudo-F_2 Population of Switchgrass

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Abstract

Switchgrass (*Panicum virgatum*) is a perennial warm-season grass that produces high biomass yield. Identification of mechanisms for genetic regulation of biomass traits has potential to facilitate genetic manipulation of switchgrass for enhancing biomass yield. The objective of this study was to identify quantitative trait loci for biomass-related traits in a pseudo-F_2 population of switchgrass derived from an upland cross with a lowland switchgrass cultivar. Plant height (HT), crown diameter (CD), and plant biomass (PB) were assessed in field studies in 2015 and 2016. Plant height was positively correlated with PB in both years but only correlated with CD in 2016. Positive correlations between CD and PB were found in both years. Six quantitative trait loci (QTLs) were detected, including three QTLs on chromosome 2b for HT (2015) or CD (2016), two 2 QTLs on chromosome 2a for CD and PB in 2016, and one QTL on chromosome 5b for CD in 2016. The logarithm of the odds scores for these QTLs ranged from 4.9 to 8.2, and percentage variance explained ranged from 7.1 to 12.9%. One QTL on chromosome 2b appeared to simultaneously control HT in 2015 and CD in 2016. Homologs of candidate genes related to cell wall development and biosynthesis, hormone regulation, and metabolism were identified within the confidence interval of these QTLs. The findings from this study indicate that these QTLs can be important signals for genetic control of switchgrass growth.

Keywords *Panicum virgatum* · QTL · Growth · Biomass traits

Introduction

Changing climate and growing energy needs are driving the production of renewable energy sources. Switchgrass (*Panicum virgatum*) is one of the candidate perennial bioenergy grasses with high water use efficiency, adaptability to marginal land, and potential for high biomass yield [1, 2]. Biomass yields of switchgrass depend on the origin of ecotypes and management practices [3]. The upland ecotypes are typically from northern latitudes, flower earlier, produce less biomass, and have higher overwintering potential compared with lowland ecotypes which are predominately from southern latitudes, flower later, produce higher biomass, and have thicker stems and fewer tillers, and typically are not cold hardy [1, 2]. Because of its considerable variability between and within ecotypes, switchgrass is adaptable across a wide variety of environments [4, 5].

Improved biomass yield is a key target for most breeding program in switchgrass [6]. It is known that tiller density and mass positively affect biomass yield in upland switchgrass that have been tested in sward conditions [7]. While plant height, tillering ability, and stem thickness can be useful in

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indirect selection for biomass yield [8, 9], plant height and stem thickness are more effective criteria for within-family biomass selection schemes in switchgrass [6]. In fact, plant biomass of spaced-planted parental genotypes can be more affected by tillering-related traits than are sward-planted progeny [10]. Although in annual species increasing plant height has been reported as correlated with increased biomass yield in sorghum (*Sorghum bicolor*) and maize (*Zea mays*) [11], tillering has been seen to be negatively correlated with plant height in rice (*Oryza sativa*) [12]. Collectively, these results demonstrate a historic challenge of using morphological traits to improving biomass yield in the plant species, including switchgrass.

Quantitative trait locus (QTL) mapping identifies statistical relationships between variation of a phenotype and underlying genetic loci. Such relationships provide insights into the genetic control of a trait of interest, as well as providing a mechanism to improve the efficiency of a breeding program. When multiple QTLs are identified as genetic components explaining variation of a trait, the trait is typically referred to as "complex." Toward this end, QTLs for tillering, plant height, flowering time, cell wall composition, and biomass yield have been detected across a variety of studies and mapping populations in switchgrass [13–20]. For example, a high number of QTL on chromosome 5a (20 QTLs) and 2a (26 QTLs) for six tillering-related traits assessed in 2 years at two locations have been identified in a lowland full-sib F_1 mapping population and a selfed population, respectively [18]. Using a full-sib mapping population derived from a cross between the lowland cultivars Kanlow and Alamo, 27 significant QTLs for 23 (out of 50) traits were discovered from evaluations within a rainout shelter facility, including QTL for biomass production co-localized on linkage group 9b across years [15]. A total of 34 and 38 main effect QTLs were detected for biomass yield and plant height in a heterozygous pseudo-F_1 population derived from a cross between lowland Alamo genotype AP13 and upland Summer genotype VS16, grown at three locations for 4 years. The percentage of variation explained for biomass yield has been reported as ranging from 3.3 to 15.3%, and from 4.3 to 17.4% for plant height [16]. Using outbred reciprocal crosses derived from lowland and upland genotypes, 33 QTLs were detected across 11 growth and disease resistance traits collected in the field and in a greenhouse [19]. Most of these studies were performed using populations derived by crosses between two lowland genotypes with a few between lowland and upland ecotypes, but all phenotypic traits were evaluated in southern US regions [14–19]. Moreover, large-effect co-localizing QTLs for multiple traits have not always been detected [19]. The complex genetic basis of important traits and genotype by environment interaction may pose an obstacle in determining multiple QTLs. To maximize QTL identifications for biomass and related traits, more genetic studies using different populations of switchgrass grown in northern environments are necessary for strengthening and extending the discovery of genetic control of biomass in this species, and to determine if these trait dissection efforts identify uniform mechanisms of biomass development across a broad geographic landscape.

As background and motivation for the current work, Tornqvist et al. [20] provided a genetic linkage map for a pseudo-F_2 population derived from tetraploid lowland and upland genotypes and identified QTLs for heading and anthesis dates across environments. In particular, the lowland genotypes used in creating this population, along with all progeny, are adaptable to northern US environments. This population would add additional values for genetic dissection of biomass and biomass component traits. Thus, in the current work reported here, we used the same pseudo-F_2 mapping population for detecting QTLs associated with plant height, crown diameter, and biomass yield of switchgrass grown under the long-day conditions found at northern latitudes. The identification of QTLs in this population allows for identification of novel QTL regions and validation of existing QTLs between southern and northern latitudes, and lend themselves to the application of marker-assisted selection in switchgrass breeding programs.

Materials and Methods

Plant Materials and Experiment Design

The population was generated from a cross of "Ellsworth" B901 (lowland) and "Summer" S041 (upland). Two F_1 progeny were crossed to create a pseudo-F_2 progeny. Newly germinated seedlings were transplanted to cone-tainers (2.5 cm diameter) and grown under natural and supplemental light in a greenhouse. Each pseudo-F_2 progeny was clonally replicated in the greenhouse, and the tillers were split multiple times before transplanting in the field. Each F_2 genotype contained one tiller per replicate, and plants were cut to the same height when planting in the field. The detailed procedure of developing the mapping population was described previously [20]. A total of 333 genotypes were planted in the summer of 2014 in Lafayette, IN, USA (40.42° N, 86.88° W). The plots were arranged in an augmented experimental design with 10 blocks, similar to the design described previously [21]. The genotypes, with one or two replicates each, were randomly assigned across the 10 blocks. The spacing between adjacent plants was 0.9 m. Plants were fertilized with 100 kg N ha^{-1} in

early spring for 2015 and 2016. Weeds were controlled by applying pre-emergent herbicide and removed manually.

Phenotypic Traits

Data for plant height, crown diameter, and plant biomass were collected in October of 2015 and 2016. Plant height was measured from the soil surface to the top of panicle. Plants were cut 10 cm from the ground level upon harvesting. Crown diameter was taken after harvesting was completed by measuring the widest point across the stubble of each individual plant. Total plant fresh weight was determined by weighing the entire plant on a mobile scale in the field. Ten tillers were randomly selected from the entire plant samples, and fresh weight was recorded. These samples were then dried at 60 °C for 3 days and weighed again for dry weight. Plant biomass was calculated based on fresh and dry weight of these tillers.

Statistical Data Analysis

A mixed model analysis of variance (PROC MIXED in SAS version 9.1; SAS Institute, 2014) was employed to identify both the significant main effects and the interactions for the fixed effects of year and genotype. Blocks were treated as a random effect. The least squares means for plant height, crown diameter, and plant biomass were calculated using PROC MIXED and were used to complete correlation and QTL analyses.

Linkage Map, QTL Mapping, and Candidate Genes

The pseudo-F_2 population was genotyped using exome capture sequencing as described previously by Evans et al. [22]. The SNPs were further filtered with respect to segregation patterns in the F_2 generation [20]. The linkage map was previously described in Tornqvist et al. [20]. Relying on R/qtl [23], a "4way" (i.e., terminology used in R/qtl for a cross between two heterozygous diploid parents) analysis or simple interval mapping using the Haley-Knott (hk) regression, with a resolution of 5 cM, was performed. The logarithm of the odds (LOD) thresholds were determined for each trait by completing a 1000 permutation test of the 'scantwo' function at the 95% confidence interval. The percentage variance explained (PVE) for QTL was estimated for each significant signal detected using the 'fitqtl' function. Orthologs of candidate genes related to plant growth and development residing within the confidence interval of each QTL were identified with switchgrass genome (v1.1). The candidate genes were selected for explaining its potential function in controlling the traits.

Results

Phenotypic Variation and Correlation

Genotype and year had significant effects on all traits (Table 1). Significant genotype × year interactions were shown for plant height (HT) and plant biomass (PB), but not for crown diameter (CD) (Table 1). Overall, HT and CD followed a normal distribution but the distribution of genotype means for PB was skewed left for both 2015 and 2016 (Fig. 1). Across the population, HT ranged from 39 to 141 cm with an average of 83.4 cm in 2015, and ranged from 27 to 120 cm with an average of 77.3 cm in 2016 (Table 2). Crown diameter ranged from 9.3 to 63.3 cm and averaged 35.2 cm in 2015, and ranged from 11.3 to 70.5 cm and averaged 38.9 cm in 2016 (Table 2). Plant biomass ranged from 46 to 2230 g with an average of 636 g in 2015, and ranged from 34 to 3618 g with an average of 1042 g in 2016 (Table 2). Plant height was positively correlated with PB ($r = 0.34$), but not CD in 2015, while, in 2016, HT was positively correlated with both CD ($r = 0.38$) and PB ($r = 0.37$) (Table 3). Positive correlations between CD and PB were found in both 2015 ($r = 0.45$) and 2016 ($r = 0.60$) (Table 3).

Linkage Map

As discussed previously, the linkage map used in this study was described in detail and used for the identification of flowering time QTLs [20]. In summary, 1223 SNPs were mapped into 18 linkage groups, with a total length of 2453 cM and an average marker interval of 2.0 cM. The coverage of the SNPs ranged from 27 SNPs on chromosome 1b to 96 SNPs on chromosome 2b.

Table 1 Results of a mixed model analysis of variance for plant height (HT), canopy diameter (CD), and plant biomass (PB) of switchgrass in 2015 and 2016 from Lafayette, IN

Trait	Interaction	df	*F* value	Significance
HT	Genotype (G)	278	1.37	**
	Year (Y)	1	9.58	**
	G × Y	266	1.38	**
CD	Genotype (G)	278	2.28	***
	Year (Y)	1	25.30	***
	G × Y	266	0.65	NS
PB	Genotype (G)	282	1.89	***
	Year (Y)	1	773.84	***
	G × Y	205	1.76	***

*Significant at 0.05 significance level

**Significant at 0.01 significance level

***Significant at 0.001 significance level

NS not significant

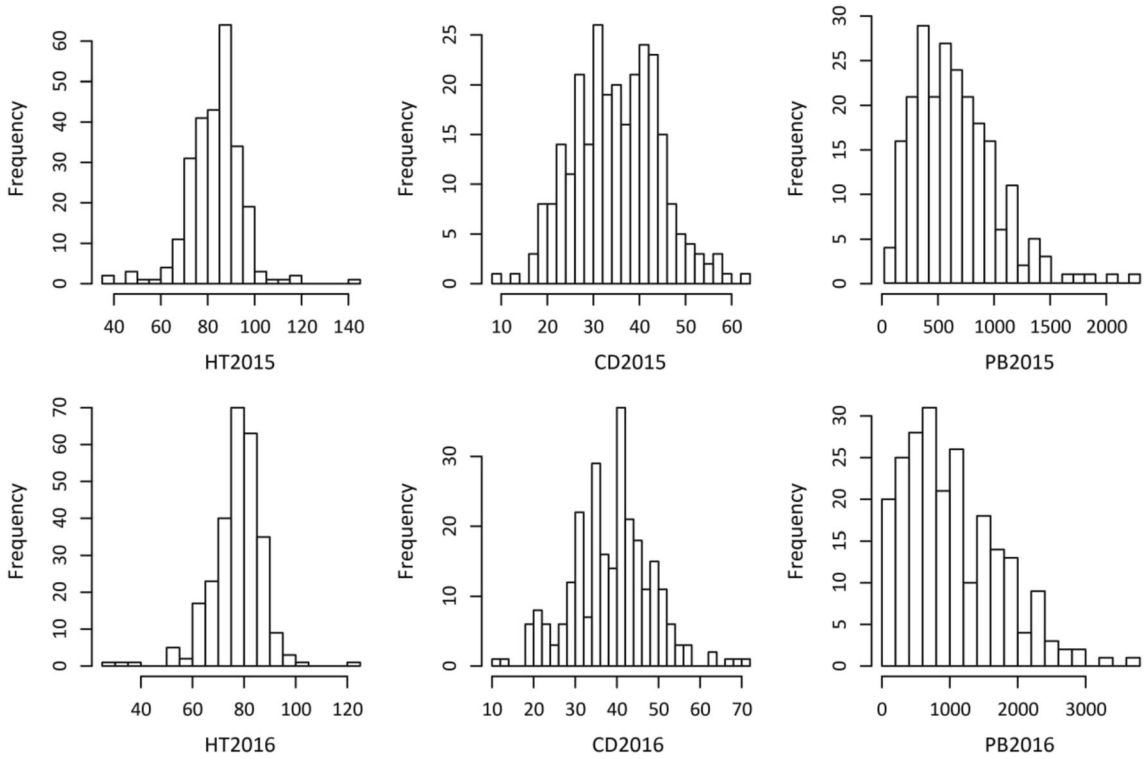

Fig. 1 Distribution of least squares genotype means for plant height (HT), crown diameter (CD), and plant biomass (PB) of switchgrass samples in both 2015 and 2016

Identification of QTLs

A total of 6 QTLs were detected for HT, CD, and PB in 2015 or 2016 (Table 4). Two QTLs for HT were identified on chromosome 2b. Of them, one QTL for *HT2015* peaked at 151 cM with LOD of 5.2 and PVE of 9.1%, while another QTL for *HT2016* peaked at 94 cM with LOD of 8.2 and PVE of 12.9% (Table 4, Fig. 2). Three QTLs for CD were detected on chromosome 2a, 2b, and 5b in 2015 or 2016. Specifically, one QTL for *CD2015* located on chromosome 2a at 31 cM with LOD of 5.1 and PVE of 8.4%. The other two QTLs were for *CD2016* on chromosome 2b at 155 cM with LOD score of 4.9 and PVE value of 7.1% and on chromosome 5b at 118 cM with LOD of 6.1 and PVE of 9.0% (Table 4, Fig. 2). The QTL for *HT2015* and *CD2016* co-localized on chromosome 2b at peak of around 151 to 155 cM. No significant QTL was detected for PB in 2015, but one significant QTL on chromosome 2a was identified in 2016 with a peak of 74 cM (Fig. 2). The LOD score was 5.1 and PVE was 9.8% for this QTL *PB2016* (Table 4).

Table 2 Descriptive statistics for switchgrass plant height (HT), crown diameter (CD), and plant biomass (PB) in both 2015 and 2016 from Lafayette, IN

Trait	Year	*N*	Mean	Standard deviation	Range
HT (cm)	2015	262	83.4	12.6	39–141
HT (cm)	2016	272	77.3	10.0	27–120
CD (cm)	2015	272	35.2	9.18	9.3–63.3
CD (cm)	2016	261	38.9	9.73	11.3–70.5
PB (g)	2015	229	636	24.7	46–2230
PB (g)	2016	228	1042	46.2	34–3618

Discussion

The overall behavior of the trait distributions for HT, CD, and PB were similar in 2015 and 2016. However, on average, the value of HT in 2015 was higher than that of 2016, while CD and PB were both higher in 2016 than in 2015. The higher average PB in 2016 was consistent with higher CD in the same year. Several factors may be responsible for increased averages between the 2 years. For example, the higher

Table 3 Pearson correlation for plant height (HT), crown diameter (CD), and plant biomass (PB) across a pseudo-F2 population of switchgrass in 2015 (low diagonal) and 2016 condition (italics, upper diagonal)

Traits	HT	CD	PB
HT		*0.38***	*0.37***
CD	0.12		*0.60***
PB	0.34***	0.45***	

***Significance at $P < 0.001$

Table 4 Location and description of quantitative trait loci (QTLs) for plant height (H), canopy diameter (CD), and plant biomass (PB) of a pseudo-F_2 switchgrass in both 2015 and 2016

QTL name	Marker	Chr.	Position (cM)	Confidence interval (cM)	QTL (bp) start	QTL (bp) end	LOD	PVE (%)
HT2015	c2b_1531081	2b	150.9	148.3–155.1	3,578,244	1,446,582	5.15	9.1
HT2016	c2b_46601236	2b	93.6	95.5–80.5	37,979,846	53,780,918	8.17	12.9
CD2015	c2a_6587916	2a	30.8	23.9–36.8	6,087,057	8,204,114	5.11	8.4
CD2016	c2b_1446582	2b	155.1	148.3–155.1	3,578,244	1,446,582	4.90	7.1
	c5b_69652421	5b	117.8	130.9–124.5	64,582,183	73,361,754	6.10	9.0
PB2016	c2a_24780883	2a	73.7	72.3–77.3	25,387,761	24,207,971	5.12	9.8

PVE percentage of phenotypic variance explained by each QTL

LOD logarithm of odds

accumulated growing degree (GDD) and monthly averaged temperature from June to October in 2016, and the higher precipitation in August 2016 when compared with 2015 may have contributed to variations in both CD and PB, especially for PB between the 2 years (Supplemental Fig. S1). Moreover, 2015 was the first year after the establishment of this specific switchgrass population, and this may have contributed to both an average lower PB and no correlation between HT and CD in 2015. The significant genotype by year interactions found in HT and PB may have caused variability in the identification of QTLs. In particular, a QTL for PB was identified in 2016, but not in 2015.

A genetic map used in this study was constructed using 1223 SNPs, with a total length of 2453 cM and SNPs mapped into 18 linkage groups as described previously [20]. The size of this map was comparable to a northern lowland genotype map with 2085 cM [13], to the Kanlow-by-Alamo pseudo-testcross map with 2200.4 cM [15], and to the reciprocal cross between upland and lowland map at 2289 cM [19]. However, our map was longer than separate male and female maps that ranged from 1462 to 1925 cM [24]. The average marker density of the current map was one marker per 2.0 cM, similar to one marker per 1.8 cM [19] but smaller than one marker per 3.5 to 4.2 cM [13, 24] observed in different populations. The current study identified six QTLs for HT, CD, or PB using a pseudo-F_2 population of switchgrass. The number of identified QTLs in this study was smaller than some previous studies [14–19]. The variations in QTL numbers in different studies could be due to varied mapping populations (e.g., F_1 or F_2 crosses made by lowland × lowland or lowland × upland

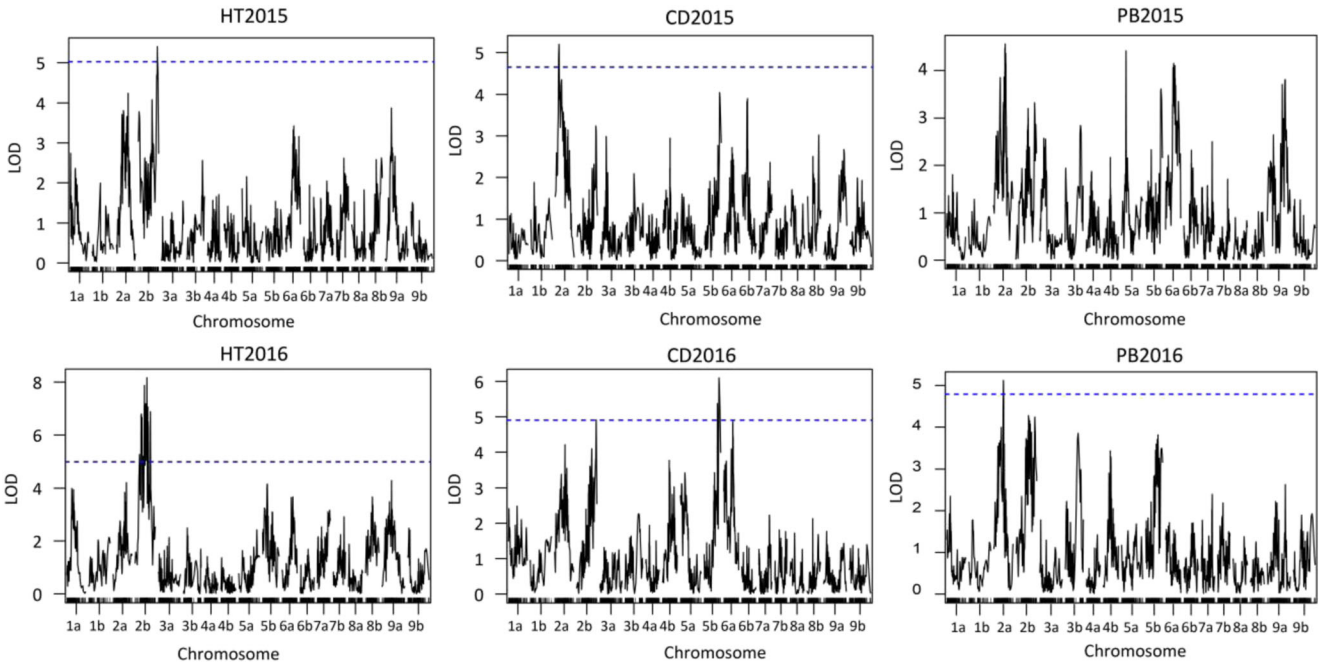

Fig. 2 Quantitative trait locus (QTL) analysis of plant height (HT), crown diameter (CD), and plant biomass (PB) of switchgrass in both 2015 and 2016. Dotted line indicated Churchill-Doerge permutation thresholds

genotypes), and a number of traits and environments assessed for the population. For example, a few studies measured more than 10 or 20 traits, and all traits were collected in two or more environments for multiple years. In this study, we only collected three biomass-related traits in one location over 2 years. This could lead to variable numbers of QTLs to be identified. Nevertheless, six QTLs identified in this study provided a novel region of QTL or verified exiting QTL signals detected in other studies. Furthermore, this number of putative QTLs would be relatively easy to manage in a marker-based breeding scheme.

One of QTLs, *HT2016*, on chromosome 2b at position 93.6 cM was considered the same QTL at position 91.5 cM for plant height discovered previously using a heterozygous pseudo-F_1 population derived from a cross between lowland Alamo and upland Summer grown in the southern USA [16]. The difference was that this *HT2016* had higher PVE for plant height (12.9%) than the previous study (8.5%) [16]. These results indicate that this QTL, controlling plant height, is robust across different populations and variable environments. In the current study, additional QTLs were detected with the same or similar effects to QTLs identified as previously reported in other switchgrass populations, but controlling different traits. On chromosome 2a, a QTL of *CD2015* at position 30.8 cM was in the same previously reported region (34.7 cM) for biomass yield [16]. Similarly, *CD2015* had higher PVE (8.4%) than the one detected in other studies (6.6%) [16]. Given the positive correlations between CD and PB, these results may indicate that these QTLs control multiple biomass and growth-related traits. In addition, the QTL, *CD2016*, on chromosome 5b at position 117.8 cM was the same QTL for flowering time at position 116.1 cM using outbred reciprocal crosses derived from lowland and upland genotypes grown in the greenhouse [19]. Notably, QTL of *PB2016* on chromosome 2a, and *HT2015* and *CD2016* on chromosome 2b were within the same region of QTLs for flowering time detected using the same genotypes of pseudo-F_2 population [20]. These results demonstrate a strong link between biomass-related traits and flowering time in switchgrass. However, *HT2015*, *CD2016*, and *PB2016* QTLs were uniquely identified in this study and these QTLs were not previously reported. Specifically, a QTL of crown width was identified on chromosome 5a at position 108 cM [15] but in our study, a QTL of *CD2016* was found on chromosome 5b at position 117.8 cM. A QTL controlling plant height was discovered on chromosome 2a at position 63.2 cM [15], but we found *HT2015* and *HT2016* both on chromosome 2b. A QTL for plant base size was found at chromosome 2a at position 96.0 cM [18] but was identified on chromosome 2a at position 20.8 cM (*CD2016*) in this study. More interestingly, the co-localized *HT2015* and *CD2016* on chromosome 2b suggest that these regions might be key targets for further improvement of switchgrass cultivars.

Plant height is primarily driven by internode elongation resulting from an increase in cell elongation and division [11]. Within the QTL regions, *HT2015* and *CD2016*, on chromosome 2b, homologs of gibberellin 2-oxidase (GA2ox) and serine/threonine-protein kinase WNK genes were identified. Gibberellin 2 oxidases (GA2oxs) catalyze the deactivation of bioactive GA or its precursors. Three GA2ox genes in switchgrass varied in their regulation patterns in roots, seedlings, and reproductive parts [25]. Overexpression of *PVGA2ox5* and *PVGA2ox9* showed dwarf phenotypes with increased tillering and reduced lignin content [25]. In addition, homologs of genes related to plant cell wall were identified in the QTL region of *HT2016* on chromosome 2b, including expansin B4 and alpha-galactosidase for loosening and extension of cell walls [26, 27] cinnamyl alcohol dehydrogenase 6 for lignin synthesis [28], xyloglucan fucosyltransferase 2 for cell wall biosynthesis [29], and glycosyl hydrolase 9B8 involved in cell wall polysaccharide degradation [30].

Crown diameter is one of the factors that contribute to overall plant growth. Homologs of the BRASSINAZOLE-RESISTANT 1 (*BZR1*) gene were found underlying the QTL, *2015CD*, on chromosome 2a. *BZR1* regulates the brassinosteriod-signaling pathway, which promotes plant growth in *Arabidopsis thaliana* and increases overall plant biomass in maize (*Zea mays*) [11, 31]. Within the confidence interval for *2016CD* on chromosome 5b, candidate genes for regulation of plant growth were identified, including cellulose synthase, elongation factor protein, and beta-6 tubulin involved in cell wall development and biosynthesis [32–34] and auxin response factor, auxin efflux carrier, gibberellin 2-oxidase 1, and cytokinin oxidase 5 related to hormone regulation, metabolism, and growth [35–37] . In addition, ZINC FINGER PROTEIN 8 (ZFP8) was identified within region of *PB2016* on chromosome 2a. This protein regulates cytokinin, abscisic acid, and gibberellic acid pathways acting as a transcription factor in *Arabidopsis* and influencing plant growth [38].

Conclusions

The current study identified QTLs that were important for plant height, crown diameter, and plant biomass of switchgrass. The utilization of a pseudo-F_2 population derived from the upland and lowland ecotype in this study allowed for more phenotypic and genotypic variations to be captured for the QTL study. Our results identified three new QTLs and verified three previously known QTLs but controlling different traits of plant growth and biomass yield. The verification of previously identified QTLs was significant, because it indicates that these chromosomal regions are robust across different mapping populations and widely different geographic regions with different growing conditions. These regions, in particular,

would be valuable targets for marker-based selection schemes to improve these three traits of switchgrass. These results provide further evidence to support confidence in marker-assisted breeding focused on improving plant biomass yield. The subsequent overlap between significant QTL regions indicates that there may be pleiotropic relationships between these traits, which is an area for future study.

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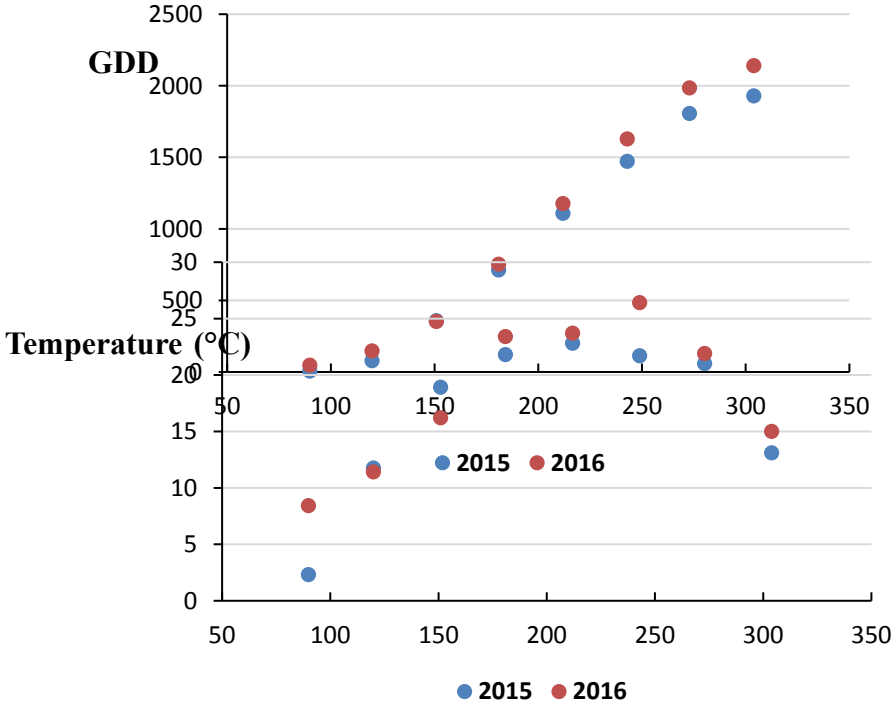

Supplemental Figure S1. The monthly accumulated growing degree (GDD) and monthly average temperatures in 2015 and 2016 of Lafayette, IN. GDD was calculated as: [(maximum temperature + minimum temperature/2)-10°C]. GDD starts accumulating after five consecutive days with average temperature >10°C (Grabowski et al., 2017).